CLAIMS

1. Recombinant screening, cloning and/or expression vector, characterized in that it replicates in mycobacteria and in that it contains:

1) a replicon which is functional in mycobacteria;

2) a selectable marker;

3) a reporter cassette comprising:

a) a multiple cloning site (polylinker),

b) optionally a transcription terminator which is active in mycobacteria, upstream of the polylinker,

c) a coding nucleotide sequence which is derived from a gene encoding a protein expression, export and/or secretion marker, said nucleotide sequence lacking its initiation codon and its regulatory sequences, and

d) a coding nucleotide sequence derived from a gene encoding a marker for the activity of promoters which are contained in the same fragment, said nucleotide sequence lacking its initiation codon.

2. Recombinant vector according to claim 1, characterized in that the coding nucleotide sequence derived from a gene encoding a protein expression, export and/or secretion marker is a coding sequence derived from the alkaline phosphatase *phoA* gene.

3. Recombinant vector according to either of claims 1 and 2, characterized in that the coding nucleotide sequence derived from a gene encoding a protein expression, export and/or secretion marker is a coding sequence of the gene for β-agarase, for the nuclease of a staphylococcus or for the β-lactamase of a mycobacterium.

4. Recombinant vector according to one of claims 1 to 3, characterized in that the coding nucleotide sequence derived from a gene encoding a marker for the activity of promoters which are contained in the same fragment is a coding sequence derived from the firefly luciferase *luc* gene.

5. Recombinant vector according to one of claims 1 to 4, characterized in that the coding nucleotide sequence derived from a gene encoding a marker for the activity of promoters which are contained in the same fragment is a coding sequence derived from the Green Fluorescent Protein *GFP* gene.

6. Recombinant vector according to one of claims 1 to 5, characterized in that the transcription terminator which is active in mycobacteria is the T4 coliphage terminator (tT4).

7. Recombinant vector according to one of claims 1 to 6, characterized in that it is a plasmid chosen from the following plasmids which have been deposited at the CNCM (Collection Nationale de Cultures de Microorganismes, Paris, France):

a) pJVEDa which was deposited at the CNCM under the No. I-1797, on 12/12/1996,

b) pJVEDb which was deposited at the CNCM under the No. I-1906, on 25 July 1997,

c) pJVEDc which was deposited at the CNCM under the No. I-1799, on 12/12/1996.

8. Recombinant vector according to one of claims 1 to 7, characterized in that it comprises at one of the cloning sites of the polylinker a nucleic acid sequence of a mycobacterium in which the detection is carried out of a polypeptide capable of being exported and/or secreted, and/or of being induced or repressed during the infection with said mycobacterium or expressed or produced constitutively, as well as the associated promoter and/or regulatory sequences which are capable of allowing or promoting the export and/or the secretion of said polypeptide, or all or part of a gene encoding said polypeptide.

9. Recombinant vector according to one of claims 1 to 8, characterized in that the mycobacterial nucleic acid sequence which it contains is obtained by physical fragmentation or by enzymatic digestion of the genomic

DNA or of the DNA which is complementary to an RNA of a mycobacterium.

10. Recombinant vector according to one of claims 1 to 9, characterized in that said mycobacterium is *M. tuberculosis*.

11. Recombinant vector according to one of claims 1 to 9, characterized in that said mycobacterium is chosen from *M. africanum*, *M. bovis*, *M. avium* or *M. leprae*.

12. Recombinant vector according to claim 10, characterized in that it is a plasmid chosen from the following plasmids which have been deposited at the CNCM:

a) p6D7 which was deposited on 28 January 1997 at the CNCM under the No. I-1814,

b) p5A3 which was deposited on 28 January 1997 at the CNCM under the No. I-1815,

c) p5F6 which was deposited on 28 January 1997 at the CNCM under the No. I-1816,

d) p2A29 which was deposited on 28 January 1997 at the CNCM under the No. I-1817,

e) pDP428 which was deposited on 28 January 1997 at the CNCM under the No. I-1818,

f) p5B5 which was deposited on 28 January 1997 at the CNCM under the No. I-1819,

g) p1C7 which was deposited on 28 January 1997 at the CNCM under the No. I-1820,

h) p2D7 which was deposited on 28 January 1997 at the CNCM under the No. I-1821,

i) p1B7 which was deposited on 31 January 1997 at the CNCM under the No. I-1843,

j) pJVED/*M. tuberculosis* which was deposited on 25 July 1997 at the CNCM under the No. I-1907,

k) pM1C25 which was deposited on 4 August 1998 at the CNCM under the No. I-2062.

13. Recombinant vector according to claim 12, characterized in that it is the plasmid pDP428 which

was deposited on 28 January 1997 at the CNCM under the No. I-1818.

14. Method of screening nucleotide sequences derived from mycobacteria in order to determine the presence of sequences corresponding to exported and/or secreted polypeptides which may be induced or repressed during the infection, their associated promoter and/or regulatory sequences which are capable in particular of allowing or promoting the export and/or secretion of said polypeptides of interest, or all or part of genes of interest encoding said polypeptides, characterized in that it uses a vector according to one of claims 1 to 13.

15. Method of screening according to claim 14, characterized in that it comprises the following steps:

a) physical fragmentation of the mycobacterial DNA sequences or their digestion with at least one defined enzyme and recovery of the fragments obtained;

b) insertion of the fragments obtained in step a) into a cloning site, which is compatible, where appropriate, with the enzyme of step a), of the polylinker of a vector according to one of claims 1 to 13;

c) if necessary, amplification of said fragments contained in the vector, for example by replication of the latter after insertion of the vector thus modified into a defined cell, preferably *E. coli*;

d) transformation of host cells with the vector amplified in step c), or in the absence of amplification, with the vector of step b);

e) culture of transformed host cells in a medium allowing the detection of the export and/or secretion marker, and/or of the promoter activity marker which is contained in the vector;

f) detection of the host cells which are positive (positive colonies) for the expression of the export and/or secretion marker, and/or of the promoter activity marker;

g) isolation of the DNA from the positive colonies and insertion of this DNA into a cell which is identical to that in step c);

h) selection of the inserts contained in the vector, allowing the production of clones which are positive for the export and/or secretion marker, and/or for the promoter activity marker;

i) isolation and characterization of the mycobacterial DNA fragments contained in these inserts, and it being possible for step i) to comprise, in addition, a step for sequencing the inserts selected.

16. Library of genomic DNA or of cDNA which is complementary to mycobacterial mRNA, characterized in that it is obtained by a method according to claim 14 and/or a method comprising steps a) and b) or a), b) and c) of the method according to claim 15.

17. Library of genomic DNA or of cDNA which is complementary to mycobacterial mRNA according to claim 16, characterized in that said mycobacterium is a pathogenic mycobacterium.

18. Library of genomic DNA or of cDNA which is complementary to mycobacterial mRNA according to claim 17, characterized in that said mycobacterium is a mycobacterium belonging to the *Mycobacterium tuberculosis* complex group.

19. Library of genomic DNA or of cDNA which is complementary to mycobacterial mRNA according to claim 18, characterized in that said mycobacterium is *Mycobacterium tuberculosis*.

20. Nucleotide sequence of mycobacterium or comprising a nucleotide sequence of mycobacterium which is capable of being selected by a method according to either of claims 14 and 15.

21. Nucleotide sequence of mycobacterium or comprising a nucleotide sequence of mycobacterium according to claim 20, characterized in that said mycobacterium is chosen from M. tuberculosis, M. bovis,

M. africanum, M. avium, M. leprae, M. paratuberculosis, M. kansassi or M. xenopi.

22. Nucleotide sequence according to either of claims 20 and 21, characterized in that it is chosen from the sequences of mycobacterial DNA fragments having the nucleic sequence SEQ ID No. 1 to SEQ ID No. 24C, SEQ ID No. 27A to SEQ ID No. 27C, SEQ ID No. 29 and SEQ ID No. 31A to SEQ ID No. 50F.

23. Nucleotide sequence of mycobacterium according to either of claims 20 and 21, characterized in that it is chosen from the sequences of mycobacterial DNA fragments having the sequence SEQ ID No. 1, SEQ ID No. 3A, SEQ ID No. 5A, SEQ ID No. 6A, SEQ ID No. 7A, SEQ ID No. 8A, SEQ ID No. 9A, SEQ ID No. 10A, SEQ ID No. 27A or SEQ ID No. 29 which are contained respectively in the vectors pDP428 (CNCM, No. I-1818), p6D7 (CNCM, No. I-1814), p5F6 (CNCM, No. I-1816), p2A29 (CNCM, No. I-1817), p5B5 (CNCM, No. I-1819), p1C7 (CNCM, No. I-1820), p2D7 (CNCM, No. I-1821), p1B7 (CNCM, No. I-1843), p5A3 (CNCM, No. I-1815) and pM1C25 (CNCM, No. I-2062).

24. Nucleotide sequence comprising the entire open reading frame of a sequence according to any one of claims 20 to 23.

25. Polynucleotide, characterized in that it comprises a polynucleotide chosen from:

a) a polynucleotide whose sequence is complementary to the sequence of a polynucleotide according to one of claims 20 to 24,

b) a polynucleotide whose sequence comprises at least 50% identity with a polynucleotide according to one of claims 20 to 24,

c) a polynucleotide which hybridizes, under high stringency conditions, with a polynucleotide sequence according to one of claims 20 to 24,

d) a fragment of at least 8 consecutive nucleotides of a polynucleotide defined according to one of claims 20 to 24 or defined in a).

26. Polypeptide, its fragments or biologically active fragments or its homologous polypeptides, which is capable of being encoded by a mycobacterial nucleotide sequence according to one of claims 20 to 25, and which is capable of being exported and/or secreted, and/or induced or repressed, or expressed constitutively during the infection.

27. Recombinant mycobacterium, characterized in that it is transformed with a recombinant vector according to one of claims 1 to 13.

28. Polynucleotide whose sequence is chosen from the nucleotide sequences having the sequence SEQ ID No. 1 to SEQ ID No. 2.

29. Polynucleotide, characterized in that it comprises a polynucleotide chosen from:

a) a polynucleotide whose sequence is chosen from the nucleotide sequences SEQ ID No. 1 to SEQ ID No. 2,

b) a polynucleotide whose nucleic sequence is the sequence between the nucleotide at position nt 964 and the nucleotide at position nt 1234, ends included, of the sequence SEQ ID No. 1,

c) a polynucleotide whose sequence is complementary to the sequence of a polynucleotide defined in a) or b),

d) a polynucleotide whose sequence exhibits at least 50% identity with a polynucleotide defined in a), b) or c),

e) a polynucleotide which hybridizes, under high stringency conditions, with a sequence of a polynucleotide defined in a), b), c) or d),

f) a fragment of at least 8 consecutive nucleotides defined in a), b), c), d) or e).

30. Polynucleotide according to one of claims 20 to 25, 28 and 29, characterized in that its nucleic sequence hybridizes with the DNA of a sequence of mycobacteria and preferably with the DNA of sequences of mycobacteria belonging to the *Mycobacterium tuberculosis* complex.

31. Polypeptide, characterized in that it is encoded by a polynucleotide sequence according to one of claims 20 to 25.

32. Polypeptide, characterized in that it comprises a polypeptide chosen from:

a) a polypeptide whose amino acid sequence is included in an amino acid sequence chosen from the amino acid sequences SEQ ID No. 1 to SEQ ID No. 24C, SEQ ID No. 27A to SEQ ID No. 28 and SEQ ID No. 30 to SEQ ID No. 50F,

b) a polypeptide which is homologous to the polypeptide defined in a),

c) a fragment of at least 5 amino acids of a polypeptide defined in a) or b),

d) a biologically active fragment of a polypeptide defined in a), b) or c).

33. Polypeptide whose amino acid sequence is included in the amino acid sequence SEQ ID No. 1 or SEQ ID No. 2, or polypeptide having the amino acid sequence SEQ ID No. 28.

34. Polynucleotide, characterized in that it encodes a polypeptide according to either of claims 32 and 33.

35. Nucleic acid sequence which can be used as a primer, characterized in that said sequence is chosen from the nucleic acid sequences of a polynucleotide according to one of claims 20 to 25, 28 to 30, and 34.

36. Nucleic acid sequence according to claim 35, characterized in that said sequence is chosen from the sequences SEQ ID No. 25 and SEQ ID No. 26.

37. Nucleic acid sequence according to either of claims 35 and 36 for the detection and/or amplification of nucleic sequences.

38. Nucleic acid sequence which can be used as a probe, characterized in that said sequence is chosen from the nucleic acid sequences according to one of claims 20 to 25, 28 to 30, and 34.

39. Nucleic acid sequence according to claim 38, characterized in that it is labeled with a radioactive compound or with a nonradioactive compound.

40. Nucleic acid sequence according to either of claims 38 and 39, characterized in that it is covalently or noncovalently immobilized on a support.

41. Nucleic acid sequence according to one of claims 38 to 40 for the detection and/or amplification of nucleic sequences.

42. Nucleic acid sequence according to one of claims 38 to 41, characterized in that said sequence is a sequence between the nucleotide at position nt 964 and the nucleotide at position nt 1234, ends included, of the sequence SEQ ID No. 1.

43. Recombinant cloning, expression and/or insertion vector, characterized in that it contains a nucleic acid sequence of the polynucleotide according to one of claims 20 to 25, 28 to 30, and 34.

44. Host cell, characterized in that it is transformed with a recombinant vector according to claim 43.

45. Host cell according to claim 44, characterized in that it is the *E. coli* strain transformed with the plasmid pDP428 deposited on 28 January 1997 at the CNCM under the No. I-1818 or transformed with the plasmid pM1C25 which was deposited on 4 August 1998 at the CNCM under the No. I-2062 or a strain of *M. tuberculosis*, *M. bovis* or *M. africanum* potentially possessing all the appropriate regulatory systems.

46. Method of preparing a polypeptide, characterized in that it uses a vector according to claim 43.

47. Recombinant polypeptide which is capable of being obtained by a method according to claim 46.

48. Hybrid polypeptide, characterized in that it comprises at least the sequence of a polypeptide according to one of claims 26, 32, 33 and 47 and a sequence of a polypeptide capable of inducing an immune response in humans or animals.

49. Hybrid polypeptide according to claim 48, characterized in that the polypeptide capable of inducing an immune response contains at least one antigenic determinant capable of inducing a humoral and/or cellular response.

50. Polynucleotide encoding a hybrid polypeptide according to either of claims 48 and 49.

51. Hybrid polypeptide according to either of claims 48 and 49, characterized in that it is a recombinant protein obtained by the expression of a polynucleotide according to claim 50.

52. Method for the *in vitro* detection of antibodies directed against a mycobacterium and preferably a bacterium of the *Mycobacterium tuberculosis* complex in a biological sample, characterized in that it comprises the following steps:

a) bringing the biological sample into contact with a polypeptide according to one of claims 26, 32, 33 and 47;

b) detection of the antigen-antibody complex formed.

53. Method for the detection of an infection by a mycobacterium and preferably a bacterium of the *Mycobacterium tuberculosis* complex in a mammal, characterized in that it comprises the following steps:

a) preparation of a biological sample containing cells of said mammal, more particularly cells of the immune system of said mammal and still more particularly T cells;

b) incubation of the biological sample of step a) with a polypeptide according to any one of claims 26, 32, 33 and 47;

c) detection of a cellular reaction indicating prior sensitization of the mammal to said polypeptide in particular cell proliferation and/or the synthesis of proteins such as interferon-gamma;

d) detection of a delayed hypersensitivity reaction or a reaction for sensitization of the mammal to said polypeptide.

54. Kit for the *in vitro* diagnosis of an infection by a mycobacterium belonging to the *Mycobacterium tuberculosis* complex, comprising:

a) a polypeptide according to one of claims 26, 32, 33 and 47;

b) where appropriate, the reagents for constituting the medium which is appropriate for the immunological reaction;

c) the reagents allowing the detection of the antigen-antibody complexes produced by the immunological reaction;

d) where appropriate, a reference biological sample (negative control) free of antibodies recognized by said polypeptide;

e) where appropriate, a reference biological sample (positive control) containing a predetermined quantity of antibodies recognized by said polypeptide.

55. Mono- or polyclonal antibodies, fragments thereof, or chimeric antibodies, characterized in that they are capable of recognizing specifically a polypeptide according to one of claims 26, 32, 33 and 47.

56. Antibody according to claim 55, characterized in that it is a labeled antibody.

57. Method for the specific detection of the presence of an antigen of a bacterium of the *Mycobacterium tuberculosis* complex in a biological sample, characterized in that it comprises the following steps:

a) bringing the biological sample into contact with an antibody according to either of claims 55 and 56;

b) detection of the antigen-antibody complex formed.

58. Kit for the specific detection of the presence of an antigen of a bacterium of the *Mycobacterium tuberculosis* complex in a biological sample, characterized in that it comprises the following components:

a) a polyclonal or monoclonal antibody according to either of claims 55 and 56;

b) a reagent for constituting the medium which is appropriate for the immunological reaction;

c) the reagents allowing the detection of the antigen-antibody complexes produced by the immunological reaction.

59. Method for the detection and rapid identification of mycobacterium and preferably of *M. tuberculosis* in a biological sample, characterized in that it comprises the following steps:

a) isolation of the DNA from the biological sample to be analyzed, or production of a cDNA from the RNA in the biological sample;

b) specific amplification of the DNA of mycobacteria belonging to the *Mycobacterium tuberculosis* complex with the aid of primers according to one of claims 35 to 37;

c) analysis of the products of amplification.

60. Method for the detection of bacteria belonging to the *Mycobacterium tuberculosis* complex in a biological sample, characterized in that it comprises the following steps:

a) bringing an oligonucleotide probe according to one of claims 38 to 42 into contact with a biological sample, the DNA contained in the biological sample having, where appropriate, been made accessible to the hybridization beforehand, under conditions allowing the hybridization of the probe with the DNA of a bacterium of the *Mycobacterium tuberculosis* complex;

b) detection of the hybrid formed between the oligonucleotide probe and the DNA of the biological sample.

61. Method for the detection of bacteria belonging to the *Mycobacterium tuberculosis* complex in a biological sample, characterized in that it comprises the following steps:

a) bringing an oligonucleotide probe according to claim 40, immobilized on a support, into contact with a biological sample, the DNA of the sample having, where appropriate, been made accessible to the hybridization beforehand, under conditions allowing the hybridization of the probe with the DNA of a bacterium of the *Mycobacterium tuberculosis* complex;

b) bringing the hybrid formed between the oligonucleotide probe immobilized on a support and the DNA contained in the biological sample, where appropriate after removal of the DNA of the biological sample which has not hybridized with the probe, into contact with a labeled oligonucleotide probe according to claim 39.

62. Method of detection according to claim 61, characterized in that, prior to step a), the DNA of the biological sample, or the cDNA obtained by reverse transcription of the RNA of the sample, is amplified with the aid of a pair of primers according to one of claims 35 to 37.

63. Method for the detection of the presence of a bacterium belonging to the *Mycobacterium tuberculosis* complex in a biological sample, characterized in that it comprises the following steps:

a) bringing the biological sample into contact with a pair of primers according to one of claims 35 to 37, the DNA contained in the sample having been, where appropriate, made accessible to hybridization beforehand, under conditions allowing hybridization of the primers with the DNA of a bacterium of the *Mycobacterium tuberculosis* complex;

b) amplification of the DNA of the bacterium of the *Mycobacterium tuberculosis* complex;

c) detection of the amplification of the DNA fragments corresponding to the fragment flanked by the primers, for example by gel electrophoresis or by means of a labeled oligonucleotide probe according to claim 39.

64. Method for the detection of the presence of a bacterium belonging to the *Mycobacterium tuberculosis* complex in a biological sample, characterized in that it comprises the following steps:

a) bringing the biological sample into contact with two pairs of primers according to one of claims 35 to 37, the DNA content in the sample having been, where appropriate, made accessible to hybridization beforehand, under conditions allowing hybridization of the primers with the DNA of a bacterium of the *Mycobacterium tuberculosis* complex;

b) amplification of the DNA of the bacterium of the *Mycobacterium tuberculosis* complex;

c) detection of the amplification of DNA fragments corresponding to the fragment flanked by said primers, for example by gel electrophoresis or by means of a labeled oligonucleotide probe according to claim 39.

65. Kit for the detection of the presence of a bacterium of the *Mycobacterium tuberculosis* complex in a biological sample, characterized in that it comprises the following components:

a) an oligonucleotide probe according to one of claims 38 to 42;

b) the reagents necessary for carrying out a hybridization reaction;

c) where appropriate, a pair of primers according to one of claims 35 to 37 as well as the reagents necessary for a reaction of amplification of the DNA (genomic DNA, plasmid DNA or cDNA) of a bacterium of the *Mycobacterium tuberculosis* complex.

66. Kit or box for the detection of the presence of a bacterium of the *Mycobacterium tuberculosis* complex in a biological sample, characterized in that it comprises the following components:

a) an oligonucleotide probe, termed capture probe, according to claim 40;

b) an oligonucleotide probe, termed revealing probe, according to one of claims 38 to 42;

c) where appropriate, a pair of primers according to claims 35 to 37 as well as the reagents necessary for a reaction of amplification of the DNA of a bacterium of the *Mycobacterium tuberculosis* complex.

67. Kit for the amplification of the DNA of a bacterium of the *Mycobacterium tuberculosis* complex present in a biological sample, characterized in that it comprises the following components:

a) a pair of primers according to one of claims 35 to 37;

b) the reagents necessary for carrying out a DNA amplification reaction;

c) optionally, a component which makes it possible to verify the sequence of the amplified fragment, more particularly an oligonucleotide probe according to one of claims 38 to 42.

68. Immunogenic composition, characterized in that it comprises one or more polypeptides according to one of claims 26, 32, 33 and 47 and/or one or more hybrid polypeptides according to one of claims 48, 49 and 51.

69. Vaccine, characterized in that it contains one or more polypeptides according to one of claims 26, 32, 33 and 47 and/or one or more hybrid polypeptides according to one of claims 48, 49 and 51, in combination with a pharmaceutically compatible vehicle and, where appropriate, one or more appropriate immunity adjuvants.

70. Vaccine intended for immunizing against a bacterial or viral infection, such as tuberculosis or heptatitis, comprising a vector according to claim 43 or a polynucleotide according to claim 50, in combination with a pharmaceutically acceptable vehicle.

71. Vaccine, characterized in that it contains one or more polynucleotide sequences according to one of claims 20 to 24 and/or one or more polynucleotides according to claim 25 in combination with a pharmaceutically compatible vehicle and, where

appropriate, one or more appropriate immunity adjuvants.

72. Method of screening molecules capable of inhibiting the growth of mycobacteria or the maintenance of mycobacteria in a host, characterized in that said molecules block the synthesis or the function of the polypeptides encoded by a nucleotide sequence according to any one of claims 20 to 24 or by a polynucleotide according to claim 25.

73. Method of screening according to claim 72, characterized in that the molecules are anti-messengers or induce the synthesis of anti-messengers.

74. Molecules capable of inhibiting the growth of mycobacteria or the maintenance of mycobacteria in a host, characterized in that said molecules are synthesized based on the structure of the polypeptides encoded by a nucleotide sequence according to any one of claims 20 to 24 or by a polynucleotide according to claim 25.